concentration of venom and various dilutions of antivenom are prepared so that the challenge dose of venom is contained in 50 µl. Controls include venom solutions incubated with physiological saline solution alone. Mixtures are incubated at 37 °C for 30 min, and aliquots of 50 µl are added to 0.2 ml of plasma or fibrinogen solution, as described. The formation or absence of clots is observed during a maximum of 30 min. The minimum volume of antivenom which completely prevents clotting is estimated and corresponds to the MCD-effective dose.

17.3.4 Neutralization of in vivo venom defibrinogenating activity

This test is a direct measure of the in vivo defibrinogenating effect of certain venoms. To measure the minimum venom defibrinogenating dose (MDD), a wide range of venom doses is selected and each dose, in a volume of 0.2 ml, is injected intravenously into 4 mice (18–20 g body weight). One hour after injection, the mice are placed under terminal general anaesthesia and bled by cardiac puncture. The blood from each animal is placed in a new glass clotting tube, left at room temperature for 1 hour and the presence/absence of a clot recorded. The MDD is defined as the minimum dose of venom that produces incoagulable blood in all mice tested within 1 hour of intravenous injection.

Antivenom neutralization of the venom component(s) responsible for in vivo defibrinogenation is estimated by incubating a challenge dose of venom, corresponding to one MDD, with different amounts of the antivenom. Controls should include venom solutions incubated with saline solution instead of antivenom. Mixtures are incubated at 37 °C for 30 min before injection of 0.2 ml by the intravenous route in groups of 4 mice (18–20 g body weight). After 1 hour, mice are bled as described above, the blood is placed in new glass clotting tubes and left undisturbed for 1 hour at room temperature, after which the presence or absence of a clot is recorded. Neutralizing ability of antivenoms is expressed as MDD-effective dose, corresponding to the minimum volume of antivenom in which the blood samples of all injected mice showed clot formation (*113*, *114*).

17.3.5 Neutralization of venom myotoxic activity

The presence of myotoxic components in a venom results in the degeneration of skeletal muscle by breaking down muscle fibres. Damage is characterized by the disruption of plasma membranes, local infiltration of inflammatory cells and oedema. Myotoxicity is characterized by the appearance of myoglobin in urine and by increments in the serum levels of muscle-derived enzymes, such as creatine kinase (CK). Myotoxic phospholipase A_2 (PLA_2) enzymes are found in a wide range of snake venoms. Some of these PLA_2s may be primarily myotoxic, or neurotoxic, or both. In addition, myotoxicity may occur as a consequence of ischaemia induced in muscle fibres by the effect of haemorrhagic venom components in the microvasculature (*115*).

Venom myotoxic activity is determined by injecting rats or mice with various doses of venom in a constant volume of 50 µl (using saline solution as diluent) into the right gastrocnemius muscle. In the case of mice, groups of 5 animals of 18–20 g body weight are used per dose. Control animals are injected with the same volume of saline solution. Tail-snip blood samples are collected at a specific time interval (3 hr in mice), and the CK activity of serum or plasma is determined using commercially-available diagnostic kits (*116*, *117*). Myotoxic activity is expressed as the minimum myotoxic dose (MMD), defined as the amount of venom that induces an increment in serum or plasma CK activity corresponding to four times the activity in serum or plasma of animals injected with saline solution alone. Myotoxicity can also be assessed by histological evaluation of muscle damage after venom injection, although this is a more expensive and more time consuming method than the CK determination.

To estimate the ability of an antivenom to neutralize venom myotoxicity, a challenge dose of venom is selected, which corresponds to 3 MMDs. The test is carried out as above, using 5 mice per group. Mixtures of a fixed concentration of venom and various dilutions of antivenom are prepared so that the challenge dose of venom is contained in 50 µl. Controls include venom solutions incubated with physiological saline solution alone. Mixtures are incubated at 37 °C for 30 min, and aliquots of 50 µl are injected into the gastrocnemius muscle, as described above. Blood samples are collected 3 hours after injection (in the case of mice) and serum or plasma CK activity is quantified. The neutralizing ability of antivenom, expressed as MMD-median effective dose (ED_{50}) is estimated as the volume of antivenom, in microlitres, which reduces the serum or plasma CK activity by 50% when compared to the activity of animals injected with venom incubated with saline solution only (*104*).

17.3.6 Neutralization of venom neurotoxic activity

Several laboratory methods for assessing venom-induced neurotoxicity have been developed (e.g. chick biventer cervicis nerve-muscle preparation (*118*, *119*); mouse hemidiaphragm phrenic nerve preparation (*120–124*), but they are difficult to perform, require costly equipment and expert technological help and are unlikely to be practicable for most antivenom producers. Mouse lethality tests are usually reliable in predicting the neutralization of neurotoxic effects of venoms.

17.4 Development of alternative assays to replace murine lethality testing

In vivo murine assays cause considerable suffering and there have been calls for the development of alternative assays to replace the standard LD_{50} and ED_{50} tests. The controversy relates to the balance between the clinical benefit to humans of preclinical testing against the cost to the experimental rodents (death, pain and distress). This issue is of considerable concern and in vivo tests should be conducted with the minimal number of animals necessary and using protocols designed to minimize pain and suffering. There are alternative tests (*124*), which reduce the need for experimental animals, use alternative non-sentient systems or use in vitro test systems. Unfortunately, such systems cannot currently replace the rodent toxicity tests. Consequently, the development of alternative methods to animal testing in the preclinical evaluation of antivenoms, should be encouraged and when live animals are absolutely necessary, anaesthesia or analgesia should be considered and evaluated to ensure that the humane benefits of anaesthesia or analgesia to the experimental animals do not invalidate the objectives of the assay by altering relevant physiological processes (*53*). The establishment of humane end-points to reduce suffering and limiting the duration of the assays to reduce the period of animal suffering is also encouraged, but would also need to be carefully evaluated to ensure the validity of the results.

17.5 Limitations of preclinical assays

It is acknowledged that the in vivo and in vitro essential and recommended preclinical tests have physiological limitations (the venom and venom/antivenom injection protocols do not represent the natural situation, and the physiological responses of rodents to envenoming and treatment may differ from those of humans). Such limitations make the rodent model of human envenoming and treatment less than ideal. Care should therefore be taken to avoid simplistic extrapolations from this assay to the clinical situation. Nevertheless, the LD_{50} and ED_{50} tests represent the methods most widely used for assessment of antivenom potency, and a number of clinical trials have demonstrated that the ED_{50} test is useful (*124*, *125*), but not infallible (*126*, *127*), at predicting the efficacy of antivenoms in the clinical setting. An additional value of these

tests is the assurance that antivenoms are manufactured with an accepted, quantifiable and uniform neutralizing potency.

17.6 Main recommendations

- **Preclinical testing of antivenoms both to determine the purification profile of the preparation and its venom(s) neutralization capacity in animal models should be a minimum regulatory requirement to be enforced by the medicines regulatory agencies.**
- **The estimation of the ability of an antivenom to neutralize the lethal activity of venom(s) (LD_{50} and ED_{50}) is the most relevant preclinical assessment and should be performed for all antivenoms.**
- **All new antivenoms, as well as existing antivenoms to be used in new geographical areas, should furthermore be assessed for their ability to eliminate specific pathologies caused by the venoms of the snakes for which the antivenom has been designed. The selection of which preclinical recommended test(s) to perform will depend on the predominant pathophysiological effects induced by the specific snake venom and be appropriately adapted for each antivenom. The recommended tests are not required for quality control assessment of subsequent batches of antivenom.**
- **Preclinical testing still relies heavily on the use of laboratory rodents and involves an unsatisfactorily high degree of suffering. The working protocols should recommend anaesthesia and analgesia to reduce suffering, where possible. Animals should be housed, fed and handled according to approved veterinary standards.**
- **Research should be promoted for the development of both refinements of the in vivo assay protocols to reduce pain and suffering of animals, and of in vitro alternatives to the in vivo assays to reduce the number of animals used in preclinical testing. The results of any modified in vivo, or new in vitro protocols, should be rigorously compared with results from existing protocols to ensure the statistical validity of the newly developed methods.**

18 CLINICAL ASSESSMENT OF ANTIVENOMS

18.1 Introduction

Antivenoms are unusual among pharmaceutical agents in that they have been used in human patients for more than 100 years with little attention paid to clinical trials of their efficacy and safety. However, since the 1970s it has been clearly demonstrated that it is possible to carry out dose-finding and randomized controlled trials in human victims of snakebite envenomings. These studies have yielded very valuable information as in the case of clinical trials of other therapeutic agents which are generally regarded as the essential basis for regulatory approval.

The standard pathway for clinical evaluation of new therapeutic products is:

- Phase I: healthy volunteer studies – detection of unanticipated adverse events
- Phase II: limited efficacy and safety studies, often dose-finding
- Phase III: full-scale clinical evaluation, often randomized controlled trials
- Phase IV: postmarketing surveillance

The appropriateness of this pathway for antivenoms depends upon a number of factors, including whether an antivenom is new or has been previously used in human patients and the practicality of undertaking such studies as well as national regulatory considerations.

The conduct of clinical studies is guided by the principles set down in the international regulations governing good clinical practice (*128–130*). These principles emphasize the responsibilities of the researcher and of the organization sponsoring the research, act to protect participants in research and ensure that the conduct of the trial is likely to lead to reliable results. Clinical trials should be registered with an appropriate registration body, prior to commencement.

18.1.1 Phase I studies

Conventional clinical studies using healthy volunteers are not appropriate in the case of antivenoms[1] because of the risk of anaphylactic and other reactions (e.g. pyrogenic or serum sickness and, rarely, hypersensitivity reactions) to volunteers. Phase I studies are primarily designed to detect unanticipated adverse events and there is extensive experience with antivenom treatment that allows a basic understanding of its pharmacokinetics.

18.1.2 Phase II and III studies

Phase II studies are usually conducted to optimize doses, establish safety of a product and give an indication of efficacy. Phase III studies are normally used to establish efficacy of a product, often in comparison with an existing product, or occasionally a placebo. Since antivenoms are so well established in the treatment of snake bite envenoming, the use of placebo controls is ethically acceptable only where there is genuine uncertainty about whether the benefit (degree of clinical improvement) from the antivenom outweighs the risk (potential rate of adverse events).

18.1.3 Phase IV studies

Phase IV studies are clinical surveillance studies that occur after market authorization of the product. In view of the difficulty in performing standard clinical trials of antivenom in some settings, this may be the only way to study safety and efficacy of an antivenom in a large number of patients.

18.2 Clinical studies of antivenoms

Although preclinical testing may be valuable in ensuring that antivenoms neutralize the venoms of interest, the complex effects of venoms in humans and the need to consider venom pharmacokinetics mean that, ultimately, the efficacy and safety of antivenoms for the treatment of human envenoming can only be determined by well designed clinical studies. Clinical studies of antivenoms primarily address three main issues:

- assessment of the optimal initial dose of antivenom;
- assessment of efficacy of the antivenom; and
- assessment of the safety of an antivenom, particularly the incidence and severity of early and late reactions.

Reaction rates for similar doses of a given batch of antivenom are unlikely to vary in different geographical locations. However, following initial preclinical testing, both efficacy and dose-finding studies may need to be repeated for a new geographical location, depending upon the

[1] Immunoglobulins derived from animal plasma.

similarity of the snake species in the new place with those where the antivenom was initially tested. If the species are similar, preclinical testing indicates good neutralization, and evidence of clinical efficacy exists in other places, postmarketing surveillance studies may be adequate.

18.2.1 Dose-finding studies

Dose-finding studies seek to establish the optimum initial dose of an antivenom required to control envenoming. The therapeutic dose of an antivenom administered by intravenous route depends on:

- the quantity of venom injected;
- the neutralizing potency of the antivenom; and
- the dose regimen.

The dose is calculated to neutralize a certain amount of venom and does not vary between adults and children. Preclinical testing may be used to estimate starting doses and these dose regimens may be evaluated in a number of ways using standard efficacy and safety end-points. Dose regimens can be assessed using prospective observational studies (*79*).

In these, the proportion of patients with good clinical outcomes (for example, restoration of blood coagulability) can be observed with different or escalating doses of antivenom.

As part of the design of the study, it is important to determine the minimum number of patients required to establish meaningful results by using sample size calculations (*131*). Results may sometimes be compared to those of previous studies (historical controls) to determine how the efficacy or safety of a newly introduced antivenom compares with previously used antivenoms (*132*). Subsequently, the minimum dose that appears to be effective can be evaluated in larger phase II trials or compared to another antivenom or a different dose in phase III randomized controlled trials.

18.2.2 Randomized controlled trials

Definitive phase III randomized controlled trials may require large numbers of patients because of considerable individual variation in the clinical manifestation of envenoming. The new antivenom is compared with the existing standard antivenom treatment or, if none exists, two different doses of the test antivenom may be compared. Placebo controls are rarely justified unless there is genuine uncertainty about the risk and benefits of antivenom treatment. In this situation, as a safeguard against unnecessary morbidity in either treatment group, a restricted sequential plan might be incorporated (*133*) which allows evaluation of results as the trial progresses, as in the early trials of therapeutic tetanus antitoxin (*134*).

To avoid bias, patients should be randomly allocated to the groups and the study should be blinded, at a minimum to those research personnel who are assessing the clinical response and ideally to both investigators and participants. There should be a calculation of the number of patients required in each trial arm to give the study sufficient statistical power. These power calculations are based on the expected difference in outcome between the treatment groups (if designed to demonstrate superiority of one treatment over another) or predefined limits of the acceptable performance compared to an existing product (if designed to demonstrate that the new antivenom is not worse than existing products (non-inferiority)).

18.2.3 Efficacy end-points for antivenom trials

The assessment criteria (end-points) used for antivenom studies should be predefined and objective. They may be clinical or assessed by laboratory investigations. Common end-points include mortality, time taken to restore blood coagulability (assessed by the 20-minute whole blood clotting test) (*135*), other laboratory parameters such as the prothrombin time, halting of bleeding or clinical improvement in neurotoxicity. Surrogate markers such as platelet count are less suitable as they may be affected by complement activation resulting from antivenom treatment itself. Patients should be observed carefully for long enough to reveal evidence of recurrent envenoming (seen particularly with short half-life Fab antivenoms) (*136*).

18.2.4 Safety end-points for antivenom trials

Because antivenoms consist of foreign proteins, adverse effects are an inevitable risk in therapy. Appropriate manufacturing steps can reduce the rate of adverse reactions. Rates of reaction are correlated with the purity of the antivenom product and the amount of protein infused. Continuous clinical observation at the bedside is necessary for several hours after treatment to detect acute reactions; late adverse reactions may occur several weeks later. Accurate reaction rates can only be assessed prospectively. Reaction rates may differ considerably between different antivenoms, but only a small proportion are life-threatening. Studies should aim to detect both early adverse events occurring at the time of, or within 24 hours of, antivenom administration (such as urticaria itching, fever, hypotension or bronchospasm) and late reactions such as serum sickness occurring between 5 and 24 days of antivenom administration (e.g. fever, urticaria, arthralgia, lymphadenopathy, proteinuria, or neuropathy).

18.2.5 Challenges in clinical testing of antivenoms

Several particular features of snakebite make clinical testing of antivenoms challenging. These features include the large variation in the consequences of envenoming between individuals making it necessary to study large number of patients, difficulties in identification of the species responsible for envenoming and the inaccessibility of areas where snakebite is sufficiently common to provide sufficient numbers of patients to study. Clinical studies may also be expensive, particularly if they need to be multicentre with the attendant additional complexity and logistics. However, despite these difficulties, a number of randomized controlled trials have been undertaken and published since 1974 (*65*, *78*, *135*, *137–142*).

18.3 Post-marketing surveillance

Phase IV studies may be of much greater importance for antivenoms than is the case for other products. A period of active post-licensing surveillance should follow:

- the introduction of a new antivenom (often a regulatory requirement);
- the introduction of an established antivenom into a new geographical area.

Although phase IV studies traditionally focus on safety, it is critical that postmarketing studies of antivenoms examine efficacy as well as the frequency of immediate or delayed side-effects. The combination of preclinical testing and postmarketing surveillance studies is a minimum acceptable clinical evaluation when an existing antivenom is used in a new region.

18.3.1 Possible approaches

Passive surveillance is currently practised by some antivenom manufacturers. However, approaches that rely upon voluntary return of questionnaires about safety and efficacy are unlikely to provide the high quality data that are necessary. There are two potential approaches to obtaining such data.

18.3.1.1 National or regional system for post-marketing surveillance

Countries using antivenoms should establish a national or regional system for the postmarketing surveillance of antivenoms. Clinicians and health workers (such as those working in poison centres) should be encouraged to report actively to national control authorities and manufacturers any unexpected lack of clinical efficacy and adverse reactions. These should include both early adverse events, occurring at the time of, or within 24 hours of, antivenom administration, and, late reactions between 5 and 24 days. The mechanism for reporting (such as the use of standardized forms), the receiving body (e.g. the national control authority), the deadline for reporting, and the type of adverse events reportable need to be clearly defined by the authority and will depend on its structure and resources. The manufacturer of the antivenom and the authorities should assess these reports and, in consultation with one another and with specialists in the field, attempt to evaluate their significance. This assessment may require the testing of products already released and the inspection of production and control facilities and local distribution channels. If an imported product is associated with adverse reactions, the manufacturer and the national control authorities both in the country of distribution and from the country of origin should be notified.

18.3.1.2 Observational studies

In certain situations, for example, the first use of an established antivenom in a new geographical area or when routine surveillance has identified safety or efficacy concerns, there is a rationale for setting up observational studies to ensure adequate efficacy and safety. In the case of first use of an established antivenom in a new geographical area, such studies should follow preclinical testing that ensures neutralization of locally important venoms. Observational studies should carefully document the clinical responses to antivenom, the clinical outcomes and the frequency of reactions in a cohort of patients (*143*).

18.3.1.3 Sentinel sites

In some settings, where postmarketing surveillance of the whole of a country may be problematic, the use of sentinel sites may allow focusing of limited resources to maximize surveillance effectiveness.

18.3.2 Responses to results of post-marketing studies

High quality postmarketing studies will allow clinicians, public health officials and manufacturers to identify antivenoms with poor effectiveness, instances of incorrect use and dosage of antivenoms and serious safety issues arising from the use of antivenoms. In some situations, these issues may be addressed by improving training of staff in the management of snakebite, but these studies may also allow identification of the use of an inappropriate antivenom (*144*).

18.4 Main recommendations

- **Preclinical and clinical testing of antivenoms has been largely neglected in the past. Despite challenges, clinical trials of antivenoms in human patients have proved feasible and useful. As far as possible, trials should adhere to the principles of WHO and International Conference on Harmonisation (ICH) good clinical practice and should measure robust end-points.**
- **National regulatory bodies should expect producers either to provide data confirming the clinical efficacy and safety of their antivenoms, against envenoming by local species of venomous snakes or, to support in-country clinical testing of these products.**
- **Prospective observational studies are fundamental to ensuring the efficacy and safety of an antivenom when first used in a new geographical region.**
- **Postmarketing surveillance studies should play a major role in the evaluation of efficacy and safety of antivenoms.**

19 ROLE OF NATIONAL REGULATORY AUTHORITIES

The WHO Guidelines for national regulatory authorities on quality assurance of biological products (*145*, *146*) state that national regulatory authorities should ensure that biological products distributed in their territories, whether imported or manufactured locally, are of good quality, safe and efficacious, and that manufacturers adhere to approved standards regarding quality assurance and good manufacturing practices. The responsibilities include the enforcement and implementation of effective national regulations, and the setting of appropriate standards and control measures.

National regulatory authorities should increasingly play a pivotal role in ensuring the quality, safety and efficacy of antivenoms. In the procedure for granting the marketing authorization for an antivenom, information on the starting material, hyperimmune animal derived plasma, the production processes and the test methods to characterize batches of the product need to be documented as part of the dossier. An example of a summary protocol of manufacturing and control of snake antivenom immunoglobulins to assist national regulatory authorities in reviewing the quality of antivenom batches is shown in Appendix 2.

Assurance of the quality, safety and efficacy of snake antivenoms involves the evaluation of information with regard to:

- the preparation of snake venom batches representative of the poisonous animals and geographical region where the antivenom will be distributed
- the control and traceability of immunized animals and animal immunization process
- the collection, storage and transport of the hyperimmune plasma
- the fractionation of the plasma and downstream processes to produce the antivenoms;
- the test methods used to control batches of the product;
- the preclinical data supporting the expected efficacy of the products for treatment of local envenomings;
- the clinical efficacy of locally manufactured or imported antivenoms against the species of snakes found in the country, through active marketing surveillance.

19.1 Impact of good manufacturing practices

Implementing the principles of GMP applied to the production of therapeutic products is acknowledged as essential for assuring the quality and safety of biological medicinal products. This approach becomes even more important and more complex due to the nature of the production process and the complexity and local specificities of snake envenomings. The implementation of an appropriate quality assurance system at all stages of manufacture, should be a pivotal element in ensuring the quality and safety of antivenoms. The following benefits derived from the compliance with GMP:

- ensures the application of quality assurance principles at all steps involved in the production of animal plasma and the fractionation process of antivenoms;
- reduces errors and technical problems at all stages of manufacture of plasma for fractionation and antivenoms;
- ensures that only products which comply with quality and safety requirements, and their marketing authorization, are released for supply;
- ensures adequate documentation and full traceability of plasma for fractionation and antivenom production;
- enables continuous improvement in production of plasma for fractionation and antivenoms;
- provides the basis for the national regulatory authorities to assess the compliance status of a manufacturer of antivenoms, either local or abroad;

19.2 Establishment licence and Inspections

The enforcement and implementation of inspection and licensing regulatory systems are fundamental tools to ensure the quality of antivenom immunoglobulins to treat snakebite envenomings. In many countries national regulatory authorities have implemented a control system based on licensing the establishments, inspecting them regularly, and enforcing the implementation of the legal requirements and applicable GMP standards. This should apply to the production of animal hyperimmune plasma for fractionation, and the manufacturing processes of the antivenoms. The inspections and control measures should be carried out by officials, representing the competent national regulatory authority. It is the responsibility of the inspector from the national regulatory authority to ensure that manufacturers adhere to the approved standards of GMP and quality assurance.

Establishments involved in all or some stages of the manufacture of antivenoms should have an establishment licence and be inspected by the competent national regulatory authority. To obtain the licence, the establishments need to show that their operation ensures compliance with a defined set of requirements supporting the safety, quality and efficacy of the antivenoms. A system control for the venoms and for the animals should be in place as part of the procedures established for the production of animal plasma for fractionation.

Inspections may follow common inspection procedures, including an opening meeting, inspection of main areas and activities for compliance with GMP requirements, a closing meeting, preparation of an inspection report and follow up of any deficiencies noted. The GMP requirements that should be covered during an inspection include verifying that all manufacturing processes and quality control tests are clearly defined and if necessary validated; all necessary resources are provided, including appropriately qualified and trained personnel, adequate premises, suitable equipment and services, appropriate materials, containers and labels, and suitable storage and transport; instructions and procedures are documented, approved,

implemented and maintained; records are kept and there is a system for handling complaints and product recall.

A thorough inspection includes the observation of staff during performance of operations and comparison with established standard operating procedures. The inspection should not only be considered as checking compliance with procedures, but also as an indirect product quality assessment by checking product-specific validation and quality control data.

A written report should summarize the main findings of the inspection including its scope, a description of the establishment, the deficiencies listed, specified and classified (e.g. as critical, major or minor), and a conclusion. The written report is sent to the manufacturer. The manufacturers are requested to notify the national regulatory authority about the specific steps which are being taken or are planned to correct the failures and to prevent their recurrence. If necessary, follow-up inspections should be performed to verify the successful implementation of specific corrective actions.

The national regulatory authority should have the authority to withdraw an establishment licence if an inspection reveals critical non-compliance with the requirements or product specifications.

AUTHORS AND ACKNOWLEDGEMENTS

These Guidelines were developed under the coordination of Dr A. Padilla, Scientist, Quality Assurance and Safety: Blood Products and Related Biologicals, World Health Organization, Geneva.

The development of this evidence-based document has been achieved thanks to the collaboration of a large number of experts supporting specific areas of the Guidelines. The experts who contributed to the drafting of the document are listed below:

The first draft was prepared by the late Dr Cassian Bon, Museum National d'Histoire Naturelle, France; Dr Thierry Burnouf, Consultant, World Health Organization; Dr Jose-Maria Gutierrez, Instituto Clodomiro Picado, Costa Rica; Dr A. Padilla, Quality Assurance and Safety of Blood Products and Related Biologicals, World Health Organization, Switzerland; Professor Kavi Ratanabanangkoon, Chulabhorn Research Institute, Thailand; and Professor DA Warrell, University of Oxford, England.

The draft was submitted for preliminary discussion to the Expert Committee on Biological Standardization (58th Meeting). This was followed by a detailed discussion within the World Health Organization Blood Regulators Nerwork (BRN), the members of which were: Dr J. Epstein, Food and Drug Administration (FDA), USA; Dr A. Farrugia, Therapeutic Goods Administration (TGA), Australia; Dr P. Ganz, Health Canada, Canada; Dr E. Griffiths, Health Canada, Canada; Dr M. Heiden, Paul-Ehrlich Institute (PEI), Germany; Dr I. Sainte Marie, Agence française de Sécurité sanitaire des Produits de Santé (Afssaps), France; Dr C. Schärer, Swissmedic, Switzerland; Dr R. Seitz, Paul-Ehrlich Institute (PEI), Germany (*Chairman*); Dr P. Zorzi, Agence française de Sécurité sanitaire des Produits de Santé (Afssaps), France. Because of the importance of these Guidelines in improving production and control of antivenoms worldwide, two World Health Organization Bi-Regional Workshops were organized in Asia and Africa, these being the Regions where snakebite envenomings constitute a major public health problem. The Workshops aimed to discuss the draft Guidelines with clinical toxinologists, manufacturers, national poison centres and regulators directly involved in manufacture and regulation of antivenoms and in the treatment of snakebite envenomings in those Regions. The Workshops were conducted by the World Health Organization with the collaboration of the following facilitators:

Dr T. Burnouf, Human Protein Process Sciences, France; Dr J.P. Chippaux, Institut de Recherche pour le Développement (IRD), Bolivia; Dr J.M. Gutierrez, Instituto Clodomiro Picado, University of Costa Rica, Costa Rica; Dr G. Müller, University of Stellenbosch, South Africa (retired); Dr A. Padilla, World Health Organization, Switzerland; Professor H.J. de Silva, Faculty of Medicine, University of Kelaniya, Sri Lanka; Professor P. Gopalakrishnakone, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; Dr R. Harrison, Liverpool School of Tropical Medicine, England; Dr D. Lalloo, Liverpool School of Tropical Medicine, England; Prof. K. Ratanabanangkoon, Chulabhorn Research Institute, Thailand; Professor D. Theakston, Liverpool School of Tropical Medicine, England (retired); Mr D. Williams, Australian Venom Research Unit/Nossal Institute for Global Health, School of Medicine, University of Melbourne, Australia.

Acknowledgements are due to the following participants of the World Health Organization Bi-Regional South-East Asian and Western Pacific Workshop on Production, Control and Regulation of Antivenoms in Jakarta, Indonesia, for their professional contributions and useful discussions:

Mrs A. Abas, Centre for Product Registration, National Pharmaceutical Control Bureau, Ministry of Health, Malaysia; Mrs S.S. Akter, Institute of Public Health, Bangladesh; Professor R. Alam, Dhaka Medical College, Bangladesh; Dr S. Amudhavalli, King Institute of Preventive Medicine, India; Mr A. Azhari, Bio Farma, Indonesia; Mr I. Baru, Drug Registration, Department of Health, Papua New Guinea; Dr B. Bissumbhar, World Health Organization, Switzerland; Dr Chhuo Meng, Poisons Centre, Calmette Hospital, Cambodia; Mr M. C. Dancel, Research Institute for Tropical Medicine, Philippines; Mr A. Fernandes, Bharat Serum and Vaccines Ltd, India; Dr S. Gnanaiah, King Institute of Preventive Medicine, India; Dr A. Gnanathasan, Department of Clinical Medicine, Faculty of Medicine, Sri Lanka; Ms L. St. Halimah, Bio Farma, Indonesia; Dr Heng Bun Kiet, Department of Drugs and Food, Ministry of Health, Cambodia; Dr T.N. Huu, Pasteur Institute, Viet Nam; Mrs W. Jariyapan, Department of Medical Sciences, Ministry of Public Health, Thailand; Dr Jing Zhang, Lanzhou Institute of Biological Products, People's Republic of China; Dr M.V. Khadilkar, Haffkine Biopharmaceutical Corporation Ltd, India; Professor S. Khomvilai, Queen Saovabha Memorial Institute, Thai Red Cross, Thailand; Dr M. Kuppusamy, VINS Bioproducts Ltd, India; Mrs D. Kusmiaty, National Agency of Drug and Food Control, Indonesia; Dr Li Jingyu, Shanghai Serum Biological Technology Co Ltd, People's Republic of China; Dr F. Malbas, Research Institute of Tropical Medicine, Muntinlupa, Philippines; Brig. Gen. S. M.A. Matin, Directorate of Drug Administration, Bangladesh; Ms M.T.S. Modina, Bureau of Food and Drugs, Philippines; Ms A. Mohamed Ariff, National Poison Center, Penang, Malaysia; Mr A. Nair, VINS Bioproducts Ltd, India; Mr N. T. Nguyen, Poison Control Centre, Viet Nam; Ms L. Nitisaporn, The Government Pharmaceutical Organization, Thailand; Dr L.R. Panganiban, National Poison Management and Control Center, University of the Philippines, Philippines; Dr V.V. Pillay, Amrita Institute of Medical Sciences & Research, India; Mr K. Ragas, CSL Biotherapies, Victoria, Australia; Dr Y. Sano, World Health Organization Regional Office for the Western Pacific, the Philippines; Dr B. Santoso, World Health Organization Regional Office for the Western Pacific, the Philippines; Dr M. Shahjahan, World Health Organization, Country Office of Indonesia; Dr Shumin Zhang, National Institute for the Control of Pharmaceutical and Biological Products, People's Republic of China; Dr L.S. Slamet, National Agency of Drug and Food Control, Indonesia; Mr J. Smith, CSL Biotherapies, Victoria Australia; Dr D. Sundari, Poison Information Center, Indonesia; Dr M. Takahashi, National Institute of Infectious Diseases, Japan; Mr B. Thapa, Ministry of Health and Population, Nepal; Dr C.L. Thapa, Ministry of Health and Population, Dumkauli Primary Health Care Center, Nepal; Dr M. Toriba, Japan Snake Institute, Japan; Mrs D. Vu Bach, Drug Registration Department, Ministry of Health, Viet Nam; Professor J. White, Women's and Children's Hospital, Australia; Dr Zhou Jing, Center for Acute Poisoning Control, Beijing, People's Republic of China.

Acknowledgements are also due to the participants at the World Health Organization Bi-Regional African and Eastern Mediterranean Workshop on Production, Control and Regulation of Antivenoms in Addis Ababa, Ethiopia for their contributions and willingness to share expertise and information:

Dr F. Aljenoobi, Food and Drug Authority, Saudi Arabia; Dr B. Allali Kouadio, Pasteur Institute, Côte d'Ivoire; Dr S. Ansar Ahmad, Ministry of Health, Pakistan; Dr M. Atef Abd-Elsalam, National Antivenom & Vaccine Production Center, Saudi Arabia; Dr S. Bah, Institut National de Recherche en Santé publique, Mali; Dr S. Bokata Masika, University of Kinshasa, Democratic Republic of Congo; Dr C.M. Baldé, Ministry of Health, Pasteur Institute, Guinea; Dr B. Bissumbhar, World Health Organization, Switzerland; Dr M. Chisale, World Health Organization, Regional Office for Africa, Congo; Professor A. Diouf, Poison Centre, Health Ministry and Prevention, Senegal; Dr N. Durfa, Federal Ministry of Health, Nigeria; Dr B. Ed Nignpense, Poisons Information & Control Centre, Ridge Hospital, Ghana; Dr M. El-Sayed Aly Ibrahim, National Regulatory Authority, Egypt;

Dr A.G. Etoundi Mballa, Hôpital Central de Yaoundé, Cameroon; Dr N.D. Fall Diop, Ministry of Health and Prevention, Senegal; Dr V. Gomwe, Medicines Control Authority, Zimbabwe; Mr M. Haladou, Ministry of Health, Niger; Dr G. Habib, Organization for Biological Products and Vaccines, Egypt; Dr C. Ilonze, National Agency for Food and Drug Administration and Control, Nigeria; Mrs N. Khalid Nomani, National Institute of Health, Pakistan; Dr F. Kolon Diallo, Direction Nationale de la Pharmacie et du Laboratoire, Ministry of Health, Guinea; Dr H. Langar, World Health Organization, Regional Office for the Eastern Mediterranean, Cairo; Mr T. Makoe, South African Vaccine Producers Ltd, South Africa; Ms M. Masanja, Food and Drugs Authority, United Republic of Tanzania; Professor A. Massougbodji, Unit of Parasitology, Benin; Dr A. Mhenni, Pasteur Institute, Tunisia; Dr A. Mohanna Mohamed, Egyptian Organization for Biological Products and Vaccines, Egypt; Dr F. Moin Siyoi, Pharmacy and Poisons Board, Ministry of Health, Kenya; Professor C. Nhachi, University of Zimbabwe Medical School, Zimbabwe; Dr G. Noreddine, Pasteur Institute, Morocco; Mr A. Okello Okonye, National Drug Authority, Uganda; Mr K.C. Yemoa, Direction des Pharmacies et du Médicament, Ministère de la Santé, Benin.

The second draft of these Guidelines was prepared, taking into consideration the information and discussions held at the abovementioned Workshops. The following experts contributed to the preparation of this draft: Dr T. Burnouf, Human Protein Process Sciences, France; Dr J. M. Gutiérrez, Instituto Clodomiro Picado, University of Costa Rica, Costa Rica; Professor Geoff Isbister, Menzies School of Health Research, Australia; Dr A. Padilla, World Health Organization, Switzerland; Professor H. J. de Silva, Faculty of Medicine, University of Kelaniya, Sri Lanka; Professor P. Gopalakrishnakone, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; Dr Rafael Otero, Programa de Ofidismo/Escorpionismo y Grupo Toxaven, Facultad de Medicina, Universidad de Antioquia Medellín, Colombia; Dr K. Regas, CSL Biotherapeutics, Australia; Dr R. Harrison, Liverpool School of Tropical Medicine, England; Dr D. Lalloo, Liverpool School of Tropical Medicine, England; Professor K. Ratanabanangkoon, Chulabhorn Research Institute, Thailand; Professor D. Theakston, Liverpool School of Tropical Medicine, England (retired); Professor Julian White, Women's and Children Hospital, Australia.

Chapter 5 and Appendix 1 of these Guidelines, on distribution of the venomous snakes of the highest medical importance worldwide, provides extremely valuable and detailed information that will assist manufacturers, regulators, public health officials, governments and nongovernmental organizations, as well as international procurement agencies, to make informed decisions with regard to the antivenoms to be considered within a particular region, country or territory. This Appendix was prepared by Mr D. Williams, Australian Venom Research Unit/Nossal Institute for Global Health, School of Medicine, University of Melbourne; Dr M. O'Shea, Australian Venom Research Unit, School of Medicine, University of Melbourne and Dr W. Wüster, School of Biological Sciences, University of Wales. The final Draft of the Appendix was reviewed by Dr D. Broadley, The National Museum of Zimbabwe, Zimbabwe; Dr J.P. Chippaux, Institut de Recherche pour le Développement (IRD), La Paz, Bolivia; Dr B. Currie, Menzies School for Health Research, Darwin, Australia; Dr J.M. Gutiérrez, Instituto Clodomiro Picado, University of Costa Rica, San José, Costa Rica; Dr U. Kuch, Biodiversität und Klima, Germany; Dr S. Seifert, USA; Professor D.A. Warrell, University of Oxford, England; Professor J. White, Women's and Children Hospital, Adelaide, Australia.

WHO Secretariat:

Dr B. Bissumbhar, Quality Assurance and Safety: Blood Products and Related Biologicals, World Health Organization, Geneva, Switzerland; Dr M. Chisale, World Health Organization, Regional Office for Africa; Dr H. Langar, World Health Organization, Regional Office for the Eastern Mediterranean; Dr F. Nafo-Trafore, World Health Organization Country Office of Ethiopia; Dr A. Padilla, Quality Assurance and Safety: Blood Products and Related Biologicals, World Health Organization, Geneva, Switzerland; Dr Y. Sano, World Health Organization, Regional Office for the Western Pacific; Dr B. Santoso, World Health Organization, Regional Office for the Western Pacific; Dr M. Shahjahan, World Health Organization, Country Office of Indonesia; Dr K. Weerasuriya, World Health Organization, Regional Office for South East Asia.

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